ANALÝZA PRVKOV V PLODOCH RODU *EPIPHYLLUM*, *HYLOCEREUS* A *OPUNTIA* (CACTACEAE) POMOCOU ENERGO-DISPERZNEJ RÖNTGENOVEJ FLUORESCENČNEJ MIKROANALÝZY

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Kľúčové slová: *Epiphyllum* Haw., *Hylocereus* (Berger) Britt., *Opuntia* Mill., energo-disperzná röntgenová fluorescenčná analýza (EDXRF/µ-XRF), stopové prvky

Úvod

Druhy rodov *Epiphyllum* Haw., *Hylocereus* (Berger) Britt. a *Opuntia* Mill. (Cactaceae – kaktusovité) sa využívajú najmä ako okrasné rastliny – kaktusy, sú však známe aj vďaka svojim jedlým plodom. Plody rodu *Hylocereus* sa nazývajú pitaya / pitahaya alebo dračie ovocie, kým plody rodu *Opuntia* voláme opunciové figy.

O farmakologickom či nutričnom význame plodov rodu *Epiphyllum* v súčasnosti nie sú dostupné žiadne štúdie. Kvety druhu *Hylocereus undatus* sa v ľudovom liečiteľstve používali na liečbu tuberkulózy, bronchitídy, mumpsu či cukrovky a na urýchlenie hojenia rán. Plody rodu *Hylocereus* sú predmetom výskumov vďaka svojej antioxidačnej, antiproliferatívnej, antimikróbnej, antidiabetickej a antihyperlipidemickej aktivite, ako aj pre svoju schopnosť uľahčovať hojenie rán[1–3]. Stonky niektorých druhov rodu *Opuntia* sú v Mexiku a Chile súčasťou výživových doplnkov. Používajú sa na prevenciu žalúdočných vredov a ako doplnková liečba *diabetes mellitus*. Plody majú potvrdené antioxidačné, antidiabetické a antihyperlipidemické účinky, pričom štúdie naznačujú aj ich ďalšie biologické aktivity, ako napr. antiproliferatívnu, antimikróbnu, protizápalovú a analgetickú. V ľudovom liečiteľstve sa niektoré druhy rodu *Opuntia* používali na liečbu gastritídy a podporu hojenia rán a popálenín[3–7].

Hlavné biologicky účinné obsahové látky prítomné v jednotlivých druhoch rodov *Epiphyllum*, *Hylocereus* a *Opuntia* sú betalaíny (fialové betakyaníny a oranžové betaxantíny), flavonoidy, fenolové kyseliny a fenylpropanoidy, terpény a steroidy, polysacharidy a mastné kyseliny. Zrelé plody všetkých troch rodov by sa potenciálne mohli použiť ako zdroj betanínu, prírodného farbiva využívaného v potravinárskom priemysle aj pod názvom E162 – cviklové farbivo[1–3,6,7].

Využitie ťažkých kovov je v priemysle rozšírené. Ich uvoľňovanie do ovzdušia, vody a pôdy narúša prirodzené rozloženie kovov v prírode. Rastliny dokážu prijímať chemické prvky z pôdy, a niektoré nežiaduce stopové prvky dokážu kumulovať až na úroveň, kedy sa stávajú toxickými. Ťažkými kovmi sa v tomto kontexte rozumejú najmä $_{48}$Cd, $_{80}$Hg a $_{82}$Pb. V širšom zmysle ide aj o ďalšie toxické prvky ako napr. $_{33}$As (pochádzajúci z určitých pesticídov) či $_{56}$Ba. Stanovenie ťažkých kovov sa všeobecne vykonáva s použitím metód buď atómovej absorpčnej spektrofotometrie (AAS) po kyslej hydrolýze vzorky, atómovej emisnej spektrometrie s indukčne viazanou plazmou (ICP-AES), hmotnostnej spektrometrie s indukčne viazanou plazmou (ICP-MS) alebo röntgenovej fluorescenčnej analýzy (XRF). Európsky liekopis / European Pharmacopoeia (Ph. Eur. 10) presne určuje limity pre jednotlivé identifikované toxické nečistoty ($_{33}$As, $_{48}$Cd, $_{28}$Cu, $_{80}$Hg, $_{28}$Ni, $_{82}$Pb)[8].

Cieľom našej práce bolo identifikovať a stanoviť chemické prvky v čerstvej šťave kaktusových plodov – *Epiphylli*, *Hylocerei* a *Opuntiae fructus* (25 vzoriek) energo-disperznou röntgenovou fluorescenčnou mikroanalýzou (EDXRF/µ-XRF).

Experimentálna časť

Rastlinný materiál

Plody rodu *Epiphyllum* Haw. pochádzali zo súkromnej záhrady v Modre, Slovensko. Plody rodu *Hylocereus* (Berger) Britt. boli nazbierané v Botanickej záhrade „Fűvészkert“ v Szegede, Maďarsko. Plody rodu *Opuntia* Mill. pochádzali z Botanickej záhrady Univerzity Komenského v Bratislave, Slovensko, a Botanickej záhrady Univerzity v Pécsi, Maďarsko. Plody boli nazbierané v septembri 2012 až 2018 a pochádzali z 5- až 10-ročných rastlín. Jednotlivé druhy uvedených rodov čeľade Cactaceae taxonomicky identifikovali systematickí botanici daných botanických záhrad. Vzorky plodov sa uskladnili na Katedre farmakognózie a botaniky (Univerzita Komenského v Bratislave, Farmaceutická fakulta, Slovensko). Vzorky (1–25) pozostávali z čerstvo lisovanej šťavy zmiešanej s metylcelulózou ako vehikulom (LACHEMA, Česká republika) v pomere 1 : 1 (suchá zmes sa následne priamo vo vzorkovnici lisovala do tabliet s priemerom 12 mm).

Prístrojové vybavenie

Philips Mini-Pal PW 4025 (MiniPal, PHILIPS ANALYTICAL, Holandsko) energo-disperzný röntgenový fluorescenčný analyzátor (EDXRF) sa použil na identifikáciu a stanovenie vzoriek[9–13]. Podmienky merania (tab. I): RTG lampa – $_{45}$Rh s $_{4}$Be okienkom, monokapilárna fokusácia, terčík $_{79}$Au, napätie 4–12 kV, prúd 100–1000 µA, atmosféra hélium alebo vzduch (1 bar), detektor s diódou Si-PIN a anódou $_{45}$Rh. Vzorky sa analyzovali počas 10–600 s; tri paralelné stanovenia sa robili. Analytické váhy (ED2245-0 CE, SARTORIUS, Nemecko).

Validácia energo-disperznej röntgenovej fluorescenčnej analýzy

Použitá analytická metóda sa čiastočne validovala v súlade s metodickými pokynmi EMA a FDA[14–16]. Zistili sa validačné charakteristiky, ako presnosť, správnosť, opakovateľnosť, špecifickosť, linearita, limit detekcie (*LOD*), limit kvantifikácie (*LOQ*), relatívna smerodajná odchýlka (*RSD*), výťažnosť a robustnosť. Série štandardov pre kalibračné krivky sa analyzovali 3-razy denne počas 3 rôznych dní, teda sa robila validácia *interday* a *intraday* na zistenie odchýlok v rámci série a medzi sériami. Presnosť a správnosť – cieľové hodnoty priemernej presnosti v rámci série a medzi sériami skúšok boli nižšie ako ± 15 % očakávanej koncentrácie. Pripravili sa dve vzorky štandardov, analyzovalo sa šesť opakovaní (n = 6) pri siedmych koncentráciách v nízkom až vysokom rozsahu (0,25–5,00 %); opakovateľnosť vyhovuje; špecifickosť bola dobrá pre prvky $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, $_{30}$Zn, rovnako aj linearita (koeficient korelácie kalibračných kriviek v koncentračnom rozsahu 0,25–5,00 %) – $_{11}$Na (NaCl): 0,9889, $_{13}$Al (Al_2O_3): 0,9896, $_{15}$P (Na_2HPO_4): 0,9948, $_{19}$K (KI): 0,9893, $_{20}$Ca ($CaCO_3$): 0,9975, $_{26}$Fe ($FeSO_4$): 0,9957, $_{27}$Co ($CoCl_2.6H_2O$): 0,9977, $_{30}$Zn (ZnO): 0,9881; *LOD* [mg/100 g] – $_{11}$Na: 0,02, $_{13}$Al: 0,03, $_{15}$P: 0,02, $_{19}$K: 0,02, $_{20}$Ca: 0,02, $_{26}$Fe: 0,02, $_{27}$Co: 0,02, $_{30}$Zn: 0,03; *LOQ* [mg/100 g] – $_{11}$Na: 0,07, $_{13}$Al: 0,09, $_{15}$P: 0,07, $_{19}$K: 0,07, $_{20}$Ca: 0,05, $_{26}$Fe: 0,06, $_{27}$Co: 0,07, $_{30}$Zn: 0,09; výťažnosť [mg/100 g] – $_{11}$Na: 0,09, $_{13}$Al: 0,09, $_{15}$P: 0,10, $_{19}$K: 0,09, $_{20}$Ca: 0,09, $_{26}$Fe: 0,09, $_{27}$Co: 0,09, $_{30}$Zn: 0.10; RSD [%] – $_{11}$Na: 40, $_{13}$Al: 12, $_{15}$P: 60, $_{19}$K: 1, $_{20}$Ca: 40, $_{26}$Fe: 2, $_{27}$Co: 14, $_{30}$Zn: 40; robustnosť sa verifikovala – napr. modifikácia prípravy vzorky, vzorkovania (zrnitosť vzoriek), homogenizácie, vlhkosti [%], teploty [°C], napätia [kV], elektrického prúdu [µA], času [s], nosného plynu a filtrov. Artefakty ako $_{28}$Ni, $_{33}$As, $_{45}$Rh a $_{79}$Au sa počas vyhodnotenia výsledkov odstránili.

Výpočet

Obsah prvkov $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, $_{30}$Zn sa vypočítal podľa požiadaviek Európskeho liekopisu / European Pharmacopoeia (Ph. Eur.). Robili sa 3 paralelné stanovenia. Údaje boli spracované pomocou programu MS Excel. Ďalšie identifikované prvky ($_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{29}$Cu, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, a $_{81}$Tl) nemohli byť stanovené v dôsledku chýbajúcich solí vhodných pre kalibráciu.

Výsledky a diskusia

Prvky analyzované v jednotlivých vzorkách boli $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{26}$Fe, $_{27}$Co, $_{29}$Cu, $_{30}$Zn, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, a $_{81}$Tl. Ich prítomnosť v jednotlivých vzorkách vykazovala značné odchýlky (tab. II).

Obsah prvkov vo vzorkách sa vypočítal pomocou kalibračných kriviek, ktoré sa pripravili pre prvky $_{11}$Na,

Tabuľka I
Podmienky merania

Vzorky + štandardy [a]	Napätie [kV]	Elektrický prúd [µA]	Čas [s]	Nosný plyn	Filter (číslo)
Vzorky 1–25	8	200	60	hélium	žiadny (5)
NaCl	4	1 000	600	hélium	žiadny (5)
Al_2O_3	5	900	120	hélium	žiadny (5)
Na_2HPO_4	5	1 000	30	hélium	žiadny (5)
KI	8	500	180	vzduch	tenký hliníkový (1)
$CaCO_3$	8	500	180	hélium	tenký hliníkový (1)
$FeSO_4$	10	100	10	vzduch	tenký hliníkový (1)
$CoCl_2.6H_2O$	10	100	120	vzduch	kaptónový (0)
ZnO	12	100	180	vzduch	kaptónový (0)

[a] Štandardy pochádzali z firmy LACHEMA, Česká republika

Tabuľka II
Prvky identifikované v čerstvej šťave z *Epiphylli*, *Hylocerei* a *Opuntiae fructus*

Vzorky [a]	Plody (farba)	Rok zberu	Identifikované prvky
1.	*Epiphyllum* sp. (fialové)	2012	$_{13}$Al, $_{15}$P, $_{19}$K, $_{27}$Co, $_{52}$Te, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{77}$Ir
2.	*Epiphyllum* sp. (ružové)	2012	$_{15}$P, $_{20}$Ca, $_{21}$Sc, $_{25}$Mn, $_{26}$Fe, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{62}$Sm, $_{63}$Eu, $_{65}$Tb, $_{77}$Ir
3.	*Epiphyllum* sp. (zelené)	2012	$_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{23}$V, $_{24}$Cr, $_{26}$Fe, $_{27}$Co, $_{35}$Br, $_{52}$Te, $_{60}$Nd, $_{61}$Pm, $_{64}$Gd, $_{65}$Tb, $_{77}$Ir
4.	*Hylocereus costaricensis* (svetloružové)	2012	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{66}$Dy, $_{75}$Re, $_{80}$Hg
5.	*Hylocereus megalanthus* (biele)	2012	$_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{31}$Ga, $_{34}$Se, $_{50}$Sn, $_{66}$Dy, $_{81}$Tl
6.	*Hylocereus undatus* (svetloružové)	2012	$_{11}$Na, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{30}$Zn, $_{50}$Sn, $_{70}$Yb
7.	*Opuntia aurea* (svetlooranžové)	2016	$_{19}$K, $_{20}$Ca, $_{27}$Co, $_{49}$In, $_{64}$Gd, $_{68}$Er, $_{70}$Yb, $_{75}$Re, $_{80}$Hg
8.	*Opuntia camanchica* (ružové)	2016	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{48}$Cd, $_{49}$In, $_{72}$Hf
9.	*Opuntia camanchica* (ružové)	2017	$_{19}$K, $_{20}$Ca, $_{27}$Co, $_{46}$Pd, $_{49}$In, $_{66}$Dy, $_{68}$Er
10.	*Opuntia camanchica* (ružové)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{49}$In, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{80}$Hg
11.	*Opuntia crinifera* (červené)	2018	$_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{21}$Sc, $_{26}$Fe, $_{35}$Br, $_{49}$In, $_{52}$Te, $_{77}$Ir
12.	*Opuntia fragilis* (fialové)	2016	$_{11}$Na, $_{19}$K, $_{20}$Ca, $_{48}$Cd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{69}$Tm, $_{70}$Yb, $_{72}$Hf
13.	*Opuntia humifusa* (tmavofialové)	2016	$_{19}$K, $_{20}$Ca, $_{32}$Ge, $_{48}$Cd, $_{49}$In, $_{65}$Tb, $_{66}$Dy
14.	*Opuntia humifusa* (tmavofialové)	2017	$_{19}$K, $_{20}$Ca, $_{27}$Co, $_{66}$Dy, $_{68}$Er, $_{70}$Yb
15.	*Opuntia humifusa* (tmavofialové)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{30}$Zn, $_{65}$Tb, $_{66}$Dy
16.	*Opuntia polyacantha* (tmavofialové)	2016	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{34}$Se, $_{48}$Cd, $_{65}$Tb, $_{71}$Lu
17.	*Opuntia tomentella* (tmavočervené)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{48}$Cd, $_{49}$In, $_{70}$Yb, $_{72}$Hf, $_{81}$Tl
18.	*Opuntia zacuapanensis* (tmavočervené)	2018	$_{15}$P, $_{19}$K, $_{20}$Ca, $_{24}$Cr, $_{26}$Fe, $_{49}$In, $_{53}$I, $_{56}$Ba, $_{61}$Pm, $_{64}$Gd, $_{65}$Tb, $_{77}$Ir
19.	*Opuntia* sp. (ružové)	2016	$_{15}$P, $_{19}$K, $_{34}$Se, $_{40}$Zr, $_{66}$Dy, $_{69}$Tm, $_{70}$Yb, $_{73}$Ta, $_{75}$Re
20.	*Opuntia* sp. (tmavofialové)	2012	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{46}$Pd, $_{49}$In, $_{67}$Ho, $_{70}$Yb, $_{76}$Os
21.	*Opuntia* sp. (purpurové)	2018	$_{11}$Na, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{30}$Zn, $_{66}$Dy, $_{70}$Yb, $_{78}$Pt
22.	*Opuntia* sp. (tmavočervené)	2015	$_{27}$Co, $_{32}$Ge, $_{46}$Pd, $_{52}$Te, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm
23.	*Opuntia* sp. (svetlooranžové)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{65}$Tb
24.	*Opuntia* sp. (oranžové)	2012	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, $_{34}$Se, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{73}$Ta, $_{80}$Hg
25.	*Opuntia* sp. (tmavooranžové)	2013	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{29}$Cu, $_{48}$Cd, $_{49}$In, $_{67}$Ho, $_{80}$Hg

[a] Vzorky 1–3, 19–25 nebolo možné botanicky identifikovať na úroveň druhu

$_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co a $_{30}$Zn (v koncentráciách 0,25–5,00 % v metylcelulóze). Obsah analyzovaných prvkov v jednotlivých vzorkách bol rôznorodý (tab. III), uvedené hodnoty sú výsledkom troch paralelných stanovení. Ďalšie identifikované prvky ($_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{29}$Cu, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg a $_{81}$Tl) nemohli byť stanovené.

Najviac zastúpeným prvkom bol draslík ($_{19}$K), ktorý bol okrem jednej vzorky prítomný vo všetkých vzorkách. Jeho koncentrácia bola 2,3 až 6,4 mg/100 g. Prítomnosť

Tabuľka III
Obsah prvkov v čerstvej šťave z *Epiphylli*, *Hylocerei* a *Opuntiae fructus*

Vzorky	Obsah [mg/100 g]							
	$_{11}$Na	$_{13}$Al	$_{15}$P	$_{19}$K	$_{20}$Ca	$_{26}$Fe	$_{27}$Co	$_{30}$Zn
1.	–	91,5	–	6,3	–	–	28,2	–
2.	–	–	382,3	–	306,6	8,8	–	–
3.	–	70,5	–	3,8	306,0	9,6	25,5	–
4.	–	–	–	5,6	304,4	8,3	–	–
5.	–	–	–	5,9	304,6	5,7	–	–
6.	131,5	–	–	5,8	305,0	4,9	–	296,2
7.	–	–	–	4,5	303,7	–	21,8	–
8.	–	–	–	4,3	305,2	3,9	–	–
9.	–	–	–	4,4	304,5	–	24,4	–
10.	–	–	–	4,5	307,5	6,3	–	–
11.	–	86,9	261,6	3,8	302,9	10,9	–	324,0
12.	–	–	–	5,7	304,1	–	–	–
13.	–	–	–	5,8	307,2	–	–	–
14.	–	–	–	5,4	303,9	–	27,6	–
15.	–	–	–	6,4	303,9	4,6	–	414,4
16.	–	–	–	5,0	306,1	9,3	–	–
17.	100,9	–	–	6,0	303,9	6,0	–	–
18.	–	–	–	4,1	305,1	2,3	–	–
19.	–	–	330,4	4,8	–	–	–	–
20.	–	–	–	4,4	301,1	3,7	–	–
21.	–	–	–	2,3	305,1	2,3	–	191,6
22.	–	–	–	3,7	–	–	32,2	–
23.	131,5	–	–	5,0	303,7	7,5	–	–
24.	–	–	–	5,7	303,6	8,0	13,9	–
25.	–	–	–	4,5	303,9	5,2	–	–

vápnika ($_{20}$Ca) bola dokázaná vo všetkých vzorkách s výnimkou troch, jeho množstvo sa pohybovalo v rozpätí od 301,1 do 307,5 mg/100 g. Tretím najviac zastúpeným prvkom bolo železo ($_{26}$Fe), prítomné v 17 vzorkách. Jeho koncentrácia bola 2,3 až 10,9 mg/100 g. Najvyššia koncentrácia sa zistila v prípade zinku ($_{30}$Zn, 414,4 mg/100 g), hoci bol prítomný iba v štyroch vzorkách (tab. III).

Vo vzorkách rodu *Epiphyllum*, nazbieraných v súkromnej záhrade v Modre, Slovensko, boli najviac zastúpenými prvkami $_{13}$Al, $_{19}$K, $_{20}$Ca, $_{26}$Fe a $_{27}$Co. Najvyššia koncentrácia bola stanovená pre $_{15}$P (382,3 mg/100 g), bol však prítomný iba v jednej vzorke.

Dostupná je iba jedna čínska štúdia, ktorá sa týka analýzy prvkov v kvetoch a stonkách druhu *Epiphyllum oxypetalum*, kde bol obsah $_{12}$Mg, $_{19}$K, $_{20}$Ca, $_{25}$Mn, $_{26}$Fe, $_{29}$Cu a $_{30}$Zn hodnotený pomocou plameňovej atómovej absorpčnej spektrometrie (FAAS). Výsledky ukázali bohatý obsah $_{25}$Mn, $_{26}$Fe, $_{29}$Cu a $_{30}$Zn v kvetoch, pričom obsah $_{19}$K bol 69,764 mg/g, približne dvojnásobný v porovnaní so stonkami[17].

Vzorky rodu *Hylocereus*, ktoré pochádzali z Botanickej záhrady v Szegede, Maďarsko, obsahovali najmä $_{19}$K, $_{20}$Ca a $_{26}$Fe, pričom $_{20}$Ca bol v týchto vzorkách nájdený v najväčšom množstve (305,0 mg/100 g).

V pitayi (*Hylocereus* sp.) pochádzajúcej z miestneho supermarketu v Pekingu, Čína, boli menej známe stopové prvky stanovené pomocou optickej emisnej spektrometrie s indukčne viazanou plazmou (ICP-OES) po mikrovlnnovej extrakcii. Táto analýza preukázala prítomnosť $_{21}$Sc (0,028 µg/g), $_{57}$La (0,423 µg/g), $_{58}$Ce (0,139 µg/g), $_{67}$Ho (0,021 µg/g) a $_{68}$Er (0,069 µg/g)[18]. Žiadny z týchto stopových prvkov nebol identifikovaný v našich vzorkách plodov rodu *Hylocereus*. Sajib a spol.[19] hodnotili obsah stopových prvkov a ťažkých kovov v plodoch *Hylocereus undatus* pochádzajúcich z miestneho trhu v meste Dháka, Bangladéš. Ich výsledky, vyjadrené v mg/100 g jedlej časti plodov, teda v dužine, boli nasledovné: 4,50 $_{11}$Na, 3,73 $_{12}$Mg, 16,14 $_{19}$K, 5,81 $_{20}$Ca, 0,02 $_{24}$Cr, 0,03 $_{25}$Mn, 0,03 $_{26}$Fe, 0,05 $_{29}$Cu, 0,44 $_{30}$Zn, pričom sa nezistila prítomnosť $_{33}$As, $_{48}$Cd, $_{80}$Hg a $_{82}$Pb. V našich vzorkách bol nižší obsah $_{19}$K a vyšší obsah $_{20}$Ca, $_{26}$Fe a $_{30}$Zn. V ďalšej štúdii autorov Hu a spol.[20] sa stanovil obsah prvkov ako $_{25}$Mn, $_{26}$Fe a $_{30}$Zn v dužine a oplodí plodov rodu *Hylocereus* pochádzajúcich z Číny pomocou plameňovej atómovej absorpčnej spektrometrie (FAAS). Dužina plodov obsahovala 23,95 μg/g $_{25}$Mn, 104,75 μg/g $_{26}$Fe a 66,40 μg/g $_{30}$Zn a oplodie obsahovalo 129,65 μg/g $_{25}$Mn, 52,15 μg/g $_{26}$Fe a 80,30 μg/g $_{30}$Zn. $_{26}$Fe bolo prítomné aj vo všetkých našich vzorkách rodu *Hylocereus* (v množstve 4,9–8,3 mg/100 g), avšak $_{30}$Zn bol dokázaný a stanovený iba v druhu *Hylocereus megalanthus*, pričom jeho koncentrácia bola omnoho vyššia (296,2 mg/100 g). $_{25}$Mn nebol prítomný v žiadnej z našich vzoriek rodu *Hylocereus*.

Existuje niekoľko štúdií, ktoré sa zaoberajú analýzou prvkov v plodoch rodu *Opuntia*. Lagunas-Solar a spol.[21] merali obsah stopových prvkov v opunciových figách (*Opuntia* sp.) pochádzajúcich z trhov a poľnohospodárskych oblastí v Kalifornii a v oblasti Tláhuac (blízko Mexico City). Ich výsledky (v mg/kg) – 3,3 $_{26}$Fe, 0,11 $_{27}$Co, 1,60 $_{28}$Ni, 3,8 $_{29}$Cu, 16,2 $_{30}$Zn a 0,050 $_{82}$Pb. Díaz-Medina a spol.[22] dokázali prítomnosť $_{11}$Na, $_{12}$Mg, $_{19}$K, $_{20}$Ca, $_{24}$Cr, $_{25}$Mn, $_{26}$Fe, $_{28}$Ni, $_{29}$Cu, $_{30}$Zn v plodoch *Opuntia ficus indica* pochádzajúcich z rôznych miest ostrova Tenerife. Stanovili obsah (v mg/kg): 6,25 $_{11}$Na, 251 $_{12}$Mg, 1 583 $_{19}$K, 263 $_{20}$Ca, 0,109 $_{24}$Cr, 3,03 $_{25}$Mn, 1,98 $_{26}$Fe, 0,285 $_{28}$Ni, 0,389 $_{29}$Cu a 2,05 $_{30}$Zn. V ďalšej analýze sa skúmala dužina plodov *Opuntia dillenii* z rôznych oblastí okresu Mysuru, Karnataka, India, a stanovil sa obsah nasledujúcich prvkov (v mg/100 g prepočítané na vysušenú drogu): 124,3 $_{11}$Na, 9,51 $_{12}$Mg, 1,16 $_{13}$Al, 29,2 $_{15}$P, 876,3 $_{19}$K, 17,6 $_{20}$Ca, 1,285 $_{25}$Mn, 5,16 $_{26}$Fe, 0,884 $_{30}$Zn, 1,27 $_{56}$Ba, okrem toho aj $_{24}$Cr, $_{29}$Cu a $_{34}$Se, ich koncentrácie však boli pod úrovňou detekcie[23]. Naše vzorky vykazovali vyšší obsah $_{11}$Na, $_{15}$P, $_{20}$Ca a $_{30}$Zn (tab. III) než koncentračné rozpätie, ktoré uvádzali spomínaní autori, a nižší obsah $_{19}$K.

Záver

V našej práci sme identifikovali a stanovili prvky v 25 vzorkách šťavy z plodov rôznych druhov rodu *Epiphyllum*, *Hylocereus* a *Opuntia*. Keďže tieto plody sú v mnohých krajinách sveta dôležitou súčasťou ľudskej potravy, je veľmi dôležité poznať obsah minerálov a stopových prvkov v nich, a taktiež úlohu, ktorú by mohli zohrať v poskytovaní živín nevyhnutných pre zdravie človeka. Podľa výsledkov nášho výskumu sa šťavy kaktusových plodov zdajú byť dobrým zdrojom fosforu ($_{15}$P), vápnika ($_{20}$Ca) a zinku ($_{30}$Zn). Aby sa pri ich konzumácii zároveň garantovala bezpečnosť konzumenta, je nevyhnutné sledovať, či sa neprekračuje obsah ťažkých kovov. Naše výsledky vyhovujú požiadavkám Európskeho liekopisu / European Pharmacopoeia (Ph. Eur. 10) pre ťažké kovy v rastlinných drogách a prípravkoch z rastlinných drog. Množstvá sledovaných ťažkých kovov v našich vzorkách boli nižšie ako limit, ktorý udáva Ph. Eur. 10, čo znamená, že náš rastlinný materiál pochádza z ekologicky vyhovujúceho prostredia a je bezpečný na použitie v potravinárskom alebo farmaceutickom priemysle.

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Sz. Czigle[a], M. Barkociová[a], T. Sovány[b], G. Regdon Jr.[b], E. Háznagy-Radnai[c], and J. Tóth[a] *([a] Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia; [b] Institute of Pharmaceutical Technology and Regulatory Affairs, Faculty of Pharmacy, University of Szeged, Szeged, Hungary, [c] Institute of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary):* **Elemental Analysis of *Epiphyllum*, *Hylocereus* and *Opuntia* (Cactaceae) Fruits by Energy-Dispersive X-ray Fluorescence Microanalysis**

Cactaceae are mostly known as ornamental plants, though they can also be used as food (e. g. Epiphylli fructus, Hylocerei fructus – pitaya / pitahaya / dragon fruit, Opuntiae fructus – Opuntia fig / tuna / prickly pear). Main phytochemical constituents responsible for their pharmacological effects are betalains, terpenes and phenolics. The subject of our work was the identification and quantification of chemical elements in Epiphylli, Hylocerei and Opuntiae fructus (25 samples) by energy-dispersive X-ray fluorescence analysis. The plant material was obtained from a garden in Modra, Slovakia (*Epiphyllum* Haw.); Botanical Garden in Szeged, Hungary [*Hylocereus* (Berger) Britt.]; Comenius University Botanical Garden in Bratislava, Slovakia, and University of Pécs Botanical Garden, Hungary (*Opuntia* Mill.). Fruits were collected in September 2012–2018. We identified and quantified these elements in the respective samples: $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, and $_{30}$Zn. The presence of these elements showed considerable variations. Other identified elements ($_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{29}$Cu, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, and $_{81}$Tl) could not be quantified due to the lack of available salts suitable for calibration.

Full text English translation available in the on-line version.

Keywords: *Epiphyllum* Haw., *Hylocereus* (Berger) Britt., *Opuntia* Mill., energy-dispersive X-ray-fluorescence analysis (EDXRF/μ-XRF), trace elements

ELEMENTAL ANALYSIS OF *EPIPHYLLUM*, *HYLOCEREUS* AND *OPUNTIA* (CACTACEAE) FRUITS BY ENERGY-DISPERSIVE X-RAY FLUORESCENCE MICROANALYSIS

Dedicated to Professor Emerita Dr. Klára Pintye-Hódi (University of Szeged, Faculty of Pharmacy, Hungary) on the occasion of her Gold Diploma.

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Keywords: *Epiphyllum* Haw., *Hylocereus* (Berger) Britt., *Opuntia* Mill., energy-dispersive X-ray-fluorescence analysis (EDXRF/μ-XRF), trace elements

Introduction

Epiphyllum Haw., *Hylocereus* (Berger) Britt. and *Opuntia* Mill. (Cactaceae) are widely used as ornamental plants. Moreover, these species are also recognised for their edible fruits. *Hylocereus* sp. is known as pitaya / pitahaya or dragon fruit, while *Opuntia* sp. as the tuna fruit or prickly pear.

To our knowledge, there are no studies regarding the pharmacological or nutritional effects of *Epiphyllum* sp. fruits. In folk medicine, flowers of *Hylocereus undatus* were used to treat tuberculosis, bronchitis, mumps or diabetes, and to speed up wounds healing. Fruits have been studied for their antioxidant, antiproliferative, antimicrobial, antidiabetic, antihyperlipidemic, and wound healing activities[1–3]. *Opuntia* sp. (stems) are used as nutritional supplements in Mexico and Chile to prevent gastric ulcers and as a complementary aid in the treatment of diabetes. Fruits indeed have antioxidant, antidiabetic and antihyperlipidemic properties. Studies also indicate other biological activities, such as antiproliferative, antimicrobial, anti-inflammatory and analgesic. In traditional medicine, *Opuntia* sp. was used to treat gastritis and to promote healing of wounds and burns[3–7].

Major bio-active compounds present in *Epiphyllum*, *Hylocereus* and *Opuntia* plants are betalains (violet betacyanins and orange betaxanthins), flavonoids, phenolic acids and fenylpropanoids, terpenes and steroids, polysaccharides and fatty acids. Ripe fruits of all three genera could potentially be used as a source of betanin, a natural colorant used in food industry under the name E162 – beetroot red[1–3,6,7].

Heavy metals are widespread in industry. When released into the air, water and soil, they disturb the naturally occurring distribution of metals. Plants extract elements from the soil in which they grow and can cumulate these undesirable trace elements up to toxic levels. Heavy metals in this context mean cadmium $_{48}$Cd, mercury $_{80}$Hg, and lead $_{82}$Pb in the first place. In a broader sense, other toxic elements, such as $_{33}$As (from certain pesticides) and $_{56}$Ba are included. Determination of heavy metals is generally performed by either atomic absorption spectrophotometry (AAS) after acid digestion of the sample, inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS) or X-ray fluorescence analysis (XRF). The European Pharmacopoeia (Ph. Eur. 10) exactly specifies limits for identified toxic impurities ($_{33}$As, $_{48}$Cd, $_{28}$Cu, $_{80}$Hg, $_{28}$Ni, $_{82}$Pb)[8].

The aim of our work was to identify and to quantify chemical elements in fresh juice from Epiphylli, Hylocerei and Opuntiae fructus (25 samples) by energy-dispersive X-ray fluorescence microanalysis (EDXRF/μ-XRF).

Experimental Parts

Plant Material

Epiphyllum Haw. fruits came from a private garden in Modra, Slovakia. *Hylocereus* (Berger) Britt. fruits were collected in the Botanical Garden "Fűvészkert" in Szeged, Hungary. *Opuntia* Mill. fruits were harvested in the Comenius University Botanical Garden in Bratislava, Slovakia, and in the University of Pécs Botanical Garden, Hungary. All fruits were collected in September 2012–2018, from 5 to 10-years-old plants. The plant material was taxonomically identified by systematic botanists (dendrologists) of the particular botanical gardens. Herbarium samples have been deposited at the Department of Pharmacognosy and Botany (Comenius University in Bratislava, Faculty of Pharmacy, Slovakia). Samples (1–25) consisted of fresh fruit juice mixed with methylcellulose as a vehicle (LACHEMA, Czech Republic) in a 1 : 1 ratio (the dry mixture was pressed into 12-mm-diameter tablets directly in the sampler).

Equipment

Philips Mini-Pal PW 4025 (MiniPal, PHILIPS ANALYTICAL, Netherlands) energy-dispersive X-ray fluorescence analyser was used to identify and quantify the samples[9–13]. Measurement conditions (Table I) applied were as follows: X-ray tube – $_{45}$Rh with $_{4}$Be window, monocapillary focusing, $_{79}$Au target, voltage 4–12 kV, electric current 100–1000 µA, 1 bar helium purge or air, and a detector with the Si-PIN diode and $_{45}$Rh anode. Samples were measured for 10–600 s and the measurements were repeated in triplicate for each sample. Analytical balance was used (ED2245-0 CE, SARTORIUS, Germany).

Validation of energy-dispersive X ray-fluorescence analysis

The analytical method was partially validated, minimum acceptance criteria were similar to the guidelines on bioanalytical method validation by EMA and FDA[14–16]. Key data are listed below to demonstrate the precision, accuracy, repeatability, specificity, linearity, limit of detection – *LOD*, limit of quantification – *LOQ*, recovery, relative standard deviation – *RSD*, and robustness. Triplicate sets of standard curve samples were analysed on 3 separate days to carry out the *inter*- and *intraday* validation. The target values for intra- and interassay precision and accuracy were less than ± 15% of the expected concentration, two standard samples were prepared, six replicates (n = 6) at seven concentrations were used in the low to high range (0.25–5.00%); repeatability was satisfactory; the used method has a good specificity for $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, and $_{30}$Zn, as well as linearity (calibration standard curve correlation coefficients in concentration range of 0.25–5.00%) – $_{11}$Na (NaCl): 0.9889, $_{13}$Al (Al_2O_3): 0.9896, $_{15}$P (Na_2HPO_4): 0.9948, $_{19}$K (KI): 0.9893, $_{20}$Ca ($CaCO_3$): 0.9975, $_{26}$Fe ($FeSO_4$): 0.9957, $_{27}$Co ($CoCl_2.6H_2O$): 0.9977, and $_{30}$Zn (ZnO): 0.9881; LOD [mg/100 g] – $_{11}$Na: 0.02, $_{13}$Al: 0.03, $_{15}$P: 0.02, $_{19}$K: 0.02, $_{20}$Ca: 0.02, $_{26}$Fe: 0.02, $_{27}$Co: 0.02, $_{30}$Zn: 003; LOQ [mg/100 g] – $_{11}$Na: 0.07, $_{13}$Al: 0.09, $_{15}$P: 0.07, $_{19}$K: 0.07, $_{20}$Ca: 0.05, $_{26}$Fe: 0.06, $_{27}$Co: 0.07, $_{30}$Zn: 009; recovery [mg/100 g] – $_{11}$Na: 0.09, $_{13}$Al: 0.09, $_{15}$P: 0.10, $_{19}$K: 0.09, $_{20}$Ca: 0.09, $_{26}$Fe: 0.09, $_{27}$Co: 0.09, $_{30}$Zn: 0.10; RSD [%] – $_{11}$Na: 40, $_{13}$Al: 12, $_{15}$P: 60, $_{19}$K: 1, $_{20}$Ca: 40, $_{26}$Fe: 2, $_{27}$Co: 14, and $_{30}$Zn: 40; robustness was verified – e. g. modification of sampling (granularity of samples), homogenisation, humidity [%], temperature [°C], voltage [kV], electric current [µA], time [s], carrier gas, filter. Artefacts, such as $_{28}$Ni, $_{33}$As, $_{45}$Rh, and $_{79}$Au were removed.

Calculation

The content of $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, and $_{30}$Zn was calculated as requested by the European Pharmacopoeia procedure. The quantification was based on the mean value of 3 parallel measurements. Quantification data were analysed using MS Excel. Other identified elements ($_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{29}$Cu, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, and $_{81}$Tl) could not be quantified due to the lack of available salts suitable for calibration.

Results and Discussion

The elements analysed in the individual samples were $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{26}$Fe, $_{27}$Co, $_{29}$Cu, $_{30}$Zn, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, and $_{81}$Tl. The presence of these elements showed considerable variations (Table II).

The content of elements in the collected samples was calculated from calibration curves. These were elaborated for $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, and $_{30}$Zn (in concentrations of 0.25–5.00% in methylcellulose). The quantification was based on the mean value of three parallel measurements. The content of the elements analysed showed differences in the respective samples (Table III). Other identified elements ($_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{29}$Cu,

Table I
Measurement conditions

Samples + standards [a]	Voltage [kV]	Electric current [µA]	Time [s]	Carrier gas	Filter (number)
Samples 1–25	8	200	60	He	none (5)
NaCl	4	1000	600	He	none (5)
Al_2O_3	5	900	120	He	none (5)
Na_2HPO_4	5	1000	30	He	none (5)
KI	8	500	180	air	Al-thin (1)
$CaCO_3$	8	500	180	He	Al-thin (1)
$FeSO_4$	10	100	10	air	Al-thin (1)
$CoCl_2.6H_2O$	10	100	120	air	kapton (0)
ZnO	12	100	180	air	kapton (0)

[a] Standards originated from LACHEMA, Czech Republic

Table II
Elements identified in fresh juice from *Epiphylli*, *Hylocerei* and *Opuntiae fructus*

Samples [a]	Fruits (colour)	Year of collection	Identified elements
1.	*Epiphyllum* sp. (violet)	2012	$_{13}$Al, $_{15}$P, $_{19}$K, $_{27}$Co, $_{52}$Te, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{77}$Ir
2.	*Epiphyllum* sp. (pink)	2012	$_{15}$P, $_{20}$Ca, $_{21}$Sc, $_{25}$Mn, $_{26}$Fe, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{62}$Sm, $_{63}$Eu, $_{65}$Tb, $_{77}$Ir
3.	*Epiphyllum* sp. (green)	2012	$_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{23}$V, $_{24}$Cr, $_{26}$Fe, $_{27}$Co, $_{35}$Br, $_{52}$Te, $_{60}$Nd, $_{61}$Pm, $_{64}$Gd, $_{65}$Tb, $_{77}$Ir
4.	*Hylocereus costaricensis* (pale pink)	2012	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{66}$Dy, $_{75}$Re, $_{80}$Hg
5.	*Hylocereus megalanthus* (white)	2012	$_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{31}$Ga, $_{34}$Se, $_{50}$Sn, $_{66}$Dy, $_{81}$Tl
6.	*Hylocereus undatus* (pale pink)	2012	$_{11}$Na, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{30}$Zn, $_{50}$Sn, $_{70}$Yb
7.	*Opuntia aurea* (pale orange)	2016	$_{19}$K, $_{20}$Ca, $_{27}$Co, $_{49}$In, $_{64}$Gd, $_{68}$Er, $_{70}$Yb, $_{75}$Re, $_{80}$Hg
8.	*Opuntia camanchica* (pink)	2016	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{48}$Cd, $_{49}$In, $_{72}$Hf
9.	*Opuntia camanchica* (pink)	2017	$_{19}$K, $_{20}$Ca, $_{27}$Co, $_{46}$Pd, $_{49}$In, $_{66}$Dy, $_{68}$Er
10.	*Opuntia camanchica* (pink)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{49}$In, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{80}$Hg
11.	*Opuntia crinifera* (red)	2018	$_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{21}$Sc, $_{26}$Fe, $_{35}$Br, $_{49}$In, $_{52}$Te, $_{77}$Ir
12.	*Opuntia fragilis* (violet)	2016	$_{11}$Na, $_{19}$K, $_{20}$Ca, $_{48}$Cd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{69}$Tm, $_{70}$Yb, $_{72}$Hf
13.	*Opuntia humifusa* (dark violet)	2016	$_{19}$K, $_{20}$Ca, $_{32}$Ge, $_{48}$Cd, $_{49}$In, $_{65}$Tb, $_{66}$Dy
14.	*Opuntia humifusa* (dark violet)	2017	$_{19}$K, $_{20}$Ca, $_{27}$Co, $_{66}$Dy, $_{68}$Er, $_{70}$Yb
15.	*Opuntia humifusa* (dark violet)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{30}$Zn, $_{65}$Tb, $_{66}$Dy
16.	*Opuntia polyacantha* (dark violet)	2016	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{34}$Se, $_{48}$Cd, $_{65}$Tb, $_{71}$Lu
17.	*Opuntia tomentella* (dark red)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{48}$Cd, $_{49}$In, $_{70}$Yb, $_{72}$Hf, $_{81}$Tl
18.	*Opuntia zacuapanensis* (dark red)	2018	$_{15}$P, $_{19}$K, $_{20}$Ca, $_{24}$Cr, $_{26}$Fe, $_{49}$In, $_{53}$I, $_{56}$Ba, $_{61}$Pm, $_{64}$Gd, $_{65}$Tb, $_{77}$Ir
19.	*Opuntia* sp. (pink)	2016	$_{15}$P, $_{19}$K, $_{34}$Se, $_{40}$Zr, $_{66}$Dy, $_{69}$Tm, $_{70}$Yb, $_{73}$Ta, $_{75}$Re
20.	*Opuntia* sp. (dark violet)	2012	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{46}$Pd, $_{49}$In, $_{67}$Ho, $_{70}$Yb, $_{76}$Os
21.	*Opuntia* sp. (purple)	2018	$_{11}$Na, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{30}$Zn, $_{66}$Dy, $_{70}$Yb, $_{78}$Pt
22.	*Opuntia* sp. (dark red)	2015	$_{27}$Co, $_{32}$Ge, $_{46}$Pd, $_{52}$Te, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm
23.	*Opuntia* sp. (pale orange)	2018	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{65}$Tb
24.	*Opuntia* sp. (orange)	2012	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, $_{34}$Se, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{73}$Ta, $_{80}$Hg
25.	*Opuntia* sp. (dark orange)	2013	$_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{29}$Cu, $_{48}$Cd, $_{49}$In, $_{67}$Ho, $_{80}$Hg

[a] Samples No 1–3, 19–25 could not be taxonomically identified by the botanists at species level

$_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, $_{81}$Tl) could not be quantified.

The most prevalent element was potassium ($_{19}$K), present in all but one sample. Its concentrations were 2.3–6.4 mg/100 g. Calcium ($_{20}$Ca) was found in all but three samples, while its concentrations ranged from 301.1 to 307.5 mg/100 g. The third most prevalent element was iron ($_{26}$Fe), present in 17 samples. Its amount was 2.3–10.9 mg/100 g. The highest concentration was observed for zinc ($_{30}$Zn, 414.4 mg/100 g), although it was present in

Table III
Elemental content in fresh juice from *Epiphylli*, *Hylocerei* and *Opuntiae fructus*

Samples	Content [mg/100g]							
	$_{11}$Na	$_{13}$Al	$_{15}$P	$_{19}$K	$_{20}$Ca	$_{26}$Fe	$_{27}$Co	$_{30}$Zn
1.	–	91.5	–	6.3	–	–	28.2	–
2.	–	–	382.3	–	306.6	8.8	–	–
3.	–	70.5	–	3.8	306.0	9.6	25.5	–
4.	–	–	–	5.6	304.4	8.3	–	–
5.	–	–	–	5.9	304.6	5.7	–	–
6.	131.5	–	–	5.8	305.0	4.9	–	296.2
7.	–	–	–	4.5	303.7	–	21.8	–
8.	–	–	–	4.3	305.2	3.9	–	–
9.	–	–	–	4.4	304.5	–	24.4	–
10.	–	–	–	4.5	307.5	6.3	–	–
11.	–	86.9	261.6	3.8	302.9	10.9	–	324.0
12.	–	–	–	5.7	304.1	–	–	–
13.	–	–	–	5.8	307.2	–	–	–
14.	–	–	–	5.4	303.9	–	27.6	–
15.	–	–	–	6.4	303.9	4.6	–	414.4
16.	–	–	–	5.0	306.1	9.3	–	–
17.	100.9	–	–	6.0	303.9	6.0	–	–
18.	–	–	–	4.1	305.1	2.3	–	–
19.	–	–	330.4	4.8	–	–	–	–
20.	–	–	–	4.4	301.1	3.7	–	–
21.	–	–	–	2.3	305.1	2.3	–	191.6
22.	–	–	–	3.7	–	–	32.2	–
23.	131.5	–	–	5.0	303.7	7.5	–	–
24.	–	–	–	5.7	303.6	8.0	13.9	–
25.	–	–	–	4.5	303.9	5.2	–	–

four samples only (Table III).

In *Epiphyllum* samples, collected from a private garden in Modra, Slovakia, the most prevalent elements were $_{13}$Al, $_{19}$K, $_{20}$Ca, $_{26}$Fe, and $_{27}$Co. The highest concentration was observed for $_{15}$P (382.3 mg/100 g), although it was present in one sample only.

To our knowledge, there is only one Chinese study regarding the elemental analysis of the genus *Epiphyllum*, where the content of $_{12}$Mg, $_{19}$K, $_{20}$Ca, $_{25}$Mn, $_{26}$Fe, $_{29}$Cu and $_{30}$Zn in the flower and stem of *Epiphyllum oxypetalum* from China was detected by flame atomic absorption spectrometry (FAAS). The results showed rich contents of $_{25}$Mn, $_{26}$Fe, $_{29}$Cu and $_{30}$Zn in the flower, while the content of $_{19}$K was 69.764 mg/g, approximately twice as much as that in the stem[17].

Hylocereus samples, which came from the Botanical Garden in Szeged, Hungary, contained mostly $_{19}$K, $_{20}$Ca and $_{26}$Fe. $_{20}$Ca was the most abundant element detected (305.0 mg/100 g) in these samples.

In a pitaya fruit (*Hylocereus* sp.) purchased from a local supermarket in Beijing, China, trace rare earth elements were determined using microwave digestion coupled with inductively coupled plasma optical emission spectrometry (ICP-OES). This analysis showed the presence of $_{21}$Sc (0.028 µg/g), $_{57}$La (0.423 µg/g), $_{58}$Ce (0.139 µg/g), $_{67}$Ho (0.021 µg/g) a $_{68}$Er (0.069 µg/g). None of these trace elements was detected in *Hylocereus* fruits in our analysis[18]. Sajib *et al.*[19] evaluated trace elements and heavy metals content in the fruits of *Hylocereus undatus* from a local market in Dhaka city, Bangladesh. Their results expressed as mg/100 g of edible portion of fruit pulps were: 4.50 of $_{11}$Na, 3.73 of $_{12}$Mg, 16.14 of $_{19}$K, 5.81 of $_{20}$Ca, 0.02 of $_{24}$Cr, 0.03 of $_{25}$Mn, 0.03 of $_{26}$Fe, 0.05 of $_{29}$Cu, 0.44 of $_{30}$Zn, while $_{33}$As, $_{48}$Cd, $_{80}$Hg and $_{82}$Pb were not detected. Our results showed lower content of $_{19}$K and higher content of $_{20}$Ca, $_{26}$Fe and $_{30}$Zn. Another study, conducted by Hu *et al.*[20], determined $_{25}$Mn, $_{26}$Fe and $_{30}$Zn trace elements content in *Hylocereus* fruit pulp and peel (from China) by flame atomic absorption spectrometry (FAAS). Results showed that the fruit pulp contained 23.95 µg/g of $_{25}$Mn, 104.75 µg/g of $_{26}$Fe, and 66.40 µg/g of $_{30}$Zn, while the peel contained 129.65 µg/g of $_{25}$Mn, 52.15 µg/g of

$_{26}$Fe, and 80.30 μg/g of $_{30}$Zn. $_{26}$Fe was also present in all our *Hylocereus* samples (ranging from 4.9 mg/100 g to 8.3 mg/100 g), while $_{30}$Zn was quantified only in *Hylocereus megalanthus;* however, its concentration was much higher (296.2 mg/100 g). $_{25}$Mn was not detected in any of our *Hylocereus* samples.

There are a few studies regarding the elemental analysis of *Opuntia* fruits. Lagunas-Solar *et al.*[21] measured trace elements in prickly pears (*Opuntia* sp.) from markets and agricultural areas in California and Tláhuac, near Mexico City. They recorded (in mg/kg) 3.3 of $_{26}$Fe, 0.11 of $_{27}$Co, 1.60 of $_{28}$Ni, 3.8 of $_{29}$Cu, 16.2 of $_{30}$Zn, and 0.050 of $_{82}$Pb. Díaz-Medina *et al.*[22] reported the presence of $_{11}$Na, $_{12}$Mg, $_{19}$K, $_{20}$Ca, $_{24}$Cr, $_{25}$Mn, $_{26}$Fe, $_{28}$Ni, $_{29}$Cu, $_{30}$Zn in *Opuntia ficus indica* fruits from different points on the Tenerife island. Their results (in mg/kg) were 6.25 of $_{11}$Na, 251 of $_{12}$Mg, 1 583 of $_{19}$K, 263 of $_{20}$Ca, 0.109 of $_{24}$Cr 3.03 of $_{25}$Mn, 1.98 of $_{26}$Fe, 0.285 of $_{28}$Ni, 0.389 of $_{29}$Cu, and 2.05 of $_{30}$Zn. In another analysis of *Opuntia dillenii* fruit pulp from various localities of Mysuru district, Karnataka, India, concentrations of different elements were determined (in mg/100 g dry weight) as follows: 124.3 $_{11}$Na, 9.51 $_{12}$Mg, 1.16 $_{13}$Al, 29.2 $_{15}$P, 876.3 $_{19}$K, 17.6 $_{20}$Ca, 1.285 $_{25}$Mn, 5.16 $_{26}$Fe, 0.884 $_{30}$Zn, 1.27 $_{56}$Ba; in addition to that, also $_{24}$Cr, $_{29}$Cu and $_{34}$Se but their concentrations were below the detectable level[23]. Our samples showed concentrations of $_{11}$Na, $_{15}$P, $_{20}$Ca and $_{30}$Zn (Table III) above the concentration range reported by previous authors was, and a lower concentration of $_{19}$K.

Conclusion

Chemical elements in 25 samples of *Epiphyllum*, *Hylocereus* and *Opuntia* fresh fruit juice were identified and quantified. As these fruits are an important part of human nutrition in many countries of the world, it is crucial to know their mineral and trace element content and the role they could play in providing nutrients essential for human health. According to our study, Cactaceae fruit juices appear to be good sources of phosphorus ($_{15}$P), calcium ($_{20}$Ca) and zinc ($_{30}$Zn). To ensure their safety for human consumption, it is necessary to track non-exceedance heavy metal content. Our results meet European Pharmacopoeia (Ph. Eur. 10) requirements for heavy metals in herbal drugs and herbal drug preparations. The amounts of monitoring heavy metals in our samples were below the limits specified in Ph. Eur. 10, which means that our plant materials can be considered to come from an ecologically clean locality and safe to be used in food and pharmaceutical industry.

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Abstract

Cactaceae are mostly known as ornamental plants, though they can also be used as food (e. g. Epiphylli fructus, Hylocerei fructus – pitaya / pitahaya / dragon fruit, Opuntiae fructus – Opuntia fig / tuna / prickly pear). Main phytochemical constituents responsible for their pharmacological effects are betalains, terpenes and phenolics. The subject of our work was the identification and quantification of chemical elements in Epiphylli, Hylocerei and Opuntiae fructus (25 samples) by energy-dispersive X-ray fluorescence analysis. The plant material was obtained from a garden in Modra, Slovakia (*Epiphyllum* Haw.); Botanical Garden in Szeged, Hungary [*Hylocereus* (Berger) Britt.]; Comenius University Botanical Garden in Bratislava, Slovakia, and University of Pécs Botanical Garden, Hungary (*Opuntia* Mill.). Fruits were collected in September 2012–2018. We identified and quantified these elements in the respective samples: $_{11}$Na, $_{13}$Al, $_{15}$P, $_{19}$K, $_{20}$Ca, $_{26}$Fe, $_{27}$Co, and $_{30}$Zn. The presence of these elements showed considerable variations. Other identified elements ($_{21}$Sc, $_{23}$V, $_{24}$Cr, $_{25}$Mn, $_{29}$Cu, $_{31}$Ga, $_{32}$Ge, $_{34}$Se, $_{35}$Br, $_{40}$Zr, $_{46}$Pd, $_{48}$Cd, $_{49}$In, $_{50}$Sn, $_{52}$Te, $_{53}$I, $_{54}$Xe, $_{56}$Ba, $_{58}$Ce, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, $_{71}$Lu, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, $_{77}$Ir, $_{78}$Pt, $_{80}$Hg, and $_{81}$Tl) could not be quantified due to the lack of available salts suitable for calibration.